INVESTIGATING THE EFFECT OF TRIPHALA EXTRACT ON LEPTIN - INDUCED INFLAMMATION IN RAW 264.7 CELL LINE

A THESIS SUBMITTED TO

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M.Sc. LIFE SCIENCES (AYURVEDA BIOLOGY)

BY

VARSHA KP

UNDER THE GUIDANCE OF

Dr. SUPRIYA BHALERAO, ASSOCIATE PROFESSOR (IRSHA)

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THE UNIVERSITY OF TRANS-DISCIPLINARY HEALTH SCIENCES AND TECHNOLOGY

Private University Established in Karnataka by ACT 35 of 2013
BENGALURU - 560064

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DECLARATION BY THE CANDIDATE

I declare that this thesis **"INVESTIGATING THE EFFECT OF TRIPHALA EXTRACT ON LEPTIN INDUCED INFLAMMATION IN RAW 264.7 CELL LINE"** submitted for the award of Master of Science to THE UNIVERSITY OF TRANS-DISCIPLINARY HEALTH SCIENCES AND TECHNOLOGY, Bengaluru, is my original work, conducted under the supervision of Dr. SUPRIYA BHALERAO. I confirm that no part of the work reported herein has been submitted for a degree or examination at any other university. References, funding, and material obtained from other sources have been duly acknowledged, and no part of this dissertation has been plagiarised.

Place: Pune **Signature of the Candidate**

Date: 15.06.2023

Name of candidate: VARSHA KP

Reg. No.: 2021MSCAB-14

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Private University Established in Karnataka by ACT 35 of 2013
BENGALURU - 560064

CERTIFICATE FROM THESIS SUPERVISOR/S

This is to certify that the work incorporated in this thesis "**INVESTIGATING THE EFFECT OF TRIPHALA EXTRACT ON LEPTIN INDUCED INFLAMMATION IN RAW 264.7 CELL LINE**" submitted by VARSHA KP was carried out under my/our supervision. No part of this thesis has been submitted for a degree or examination at any other university. References, help and material obtained from other sources have been duly acknowledged. I confirm the originality of the work and that there is no plagiarism in any part of the thesis.

Name: **Dr.Supriya Bhalerao**

Designation: Associate professor & Head
Interactive Research School for Health Affairs (IRSHA), Bharati Vidyapeeth Deemed to be University, Pune

Signature,
Date- 03/07/2023

(Signature attached)

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INTERACTIVE RESEARCH SCHOOL FOR HEALTH AFFAIRS
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Bhalerao

Dr. Supriya Bhalerao
Associate Professor

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Additionally, I would like to acknowledge the unwavering support and motivation from my colleagues throughout this research journey. Their encouragement, discussions, and shared experiences significantly enriched my understanding and helped overcome various challenges encountered during the course of this study.

SUMMARY

This study aimed to investigate the effects of leptin and Triphala extract on inflammation using the RAW 264.7 cell line as a model. Leptin, a hormone produced by adipocytes, has been associated with various physiological functions, including inflammation. In our study, we confirmed that leptin induces inflammation in the RAW 264.7 cells, leading to increased production of nitric oxide (NO). However, the administration of Triphala extract demonstrated significant anti-inflammatory effects. When combined with leptin, Triphala effectively reduced the inflammation induced by leptin, as indicated by decreased NO release. These findings highlight the potential of Triphala as a natural anti-inflammatory agent.

The experiments were conducted using the RAW 264.7 cell line, which serves as a suitable model for studying monocyte/macrophage-like cells. Our results showed a dose-dependent response to leptin, with higher concentrations resulting in more pronounced inflammation. Nonetheless, Triphala supplementation successfully mitigated this inflammation, even at lower concentrations of leptin.

This study advances the field of research by demonstrating the efficacy of Triphala as a medicinal agent for treating inflammation. The results emphasize Triphala's capacity to lessen the pro-inflammatory effects of leptin, pointing to its applicability in treating inflammatory diseases. Overall, this study clarifies the role of Triphala in the study of inflammation and its anti-inflammatory characteristics.

PERSONAL REFLECTION

This period of time has been incredibly rewarding and has allowed me to experience significant personal and professional growth.

In terms of personal growth, I have noticed a remarkable improvement in my self-confidence. I have become more assured in my abilities and have developed a character that is less prone to stress and tension. This newly acquired conviction has been crucial in helping me overcome the challenges that I came across. I started embracing new opportunities with a positive mindset.

Professionally, I have improved my communication skills, enabling me to express ideas effectively. I have also strengthened my problem-solving and decision-making abilities, approaching tasks with clarity and strategy. Moreover, I have learned the value of efficient time management, balancing responsibilities, and meeting deadlines successfully.

I gained hands-on experience in cell culture techniques in an animal tissue culture laboratory. This starts from maintaining cell lines in proper sterile conditions to conducting biochemistry-based assays like MTT and NO assays.

I had an opportunity to conduct an ongoing clinical study in the orthopedic unit, at Bharathi Hospital Pune. Here, I got experience in Randomization, screening, recruitment, the follow-up process, and documentation (MS Excel and Case report file). Also, I got a chance to learn a new language Marathi, which helped me interact with locals efficiently during the clinical study.

By delivering multiple scientific papers, I was able to broaden my knowledge and hone my presentation skills. Additionally, I had the opportunity to contribute to data collection and documentation for an app called Ayurprameha.

Moreover, I had the privilege of obtaining a Good Clinical Practice certificate, which provided me with a comprehensive understanding of clinical research, including research design, ethical considerations, and protocols.

Balancing the demands of the research project, laboratory work, and academic commitments has taught me the importance of effective time allocation and prioritization. Through careful planning and organization, I have been able to meet deadlines and deliver quality work.

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1. INTRODUCTION

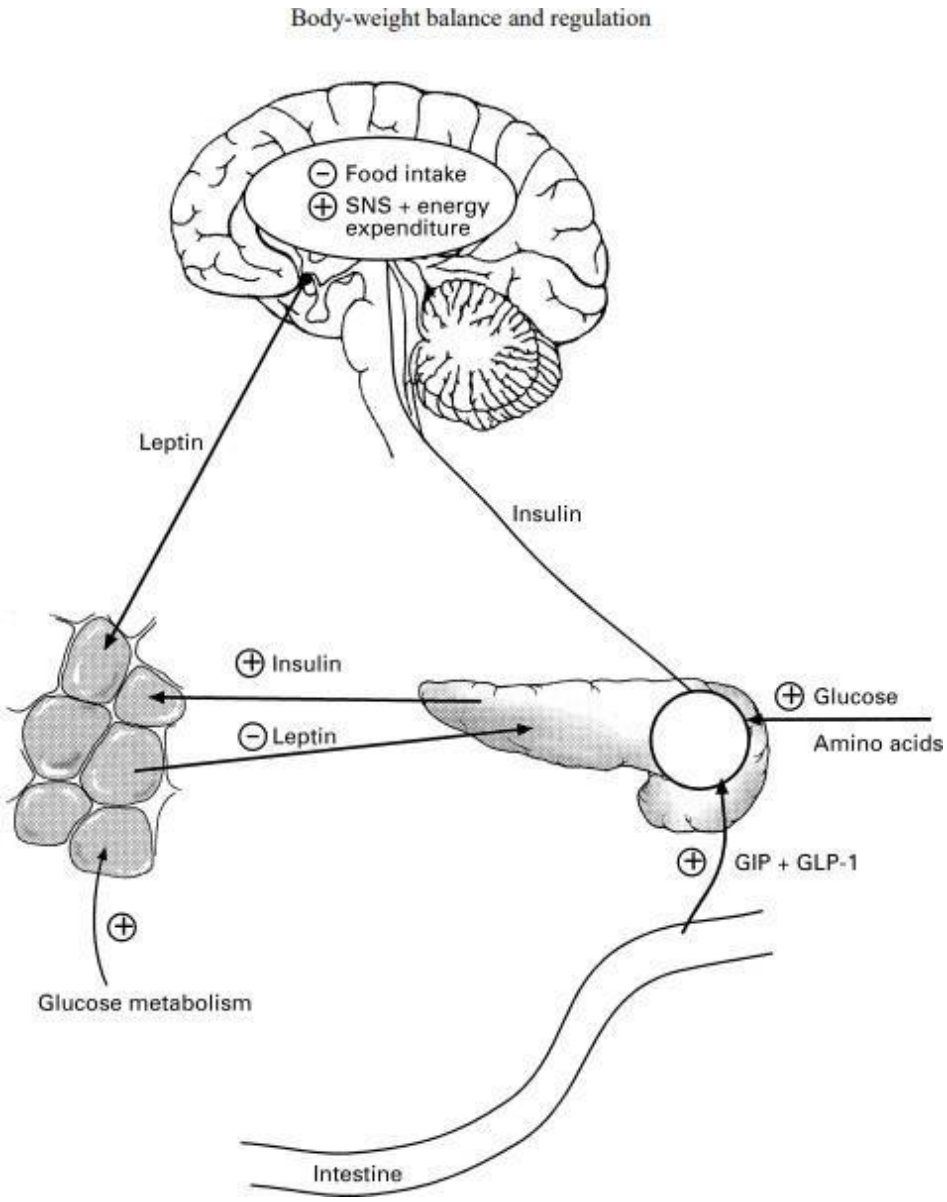
1.1 Inflammation

Inflammation, as an immune response, plays a crucial role in maintaining tissue homeostasis. It serves as an adaptive mechanism when other mechanisms are insufficient, resulting in either acute or chronic inflammation. The pathogenic potential of chronic inflammation serves as a common underlying factor in multifactorial diseases such as cardiovascular disease and type 2 diabetes. Recent interest has focused on the discovery of new inflammatory mediators, as there is a possibility that metabolic, autoimmune, or infectious diseases may induce leptin resistance at the central level. This resistance can lead to chronic inflammatory states that exacerbate obesity through increased leptin levels and perpetuate inflammation (Pérez-Pérez et al., 2020)

1.2 Leptin

Leptin, a peptide hormone produced primarily by adipose tissue, is involved in the regulation of body weight and energy balance. It exerts its effects through a complex network of signaling pathways in both the central nervous system and peripheral tissues. Leptin levels in the bloodstream, which typically range around 16 ng/mL, are correlated with the amount of energy preserved in adipose tissue and are higher in individuals with obesity compared to leaner individuals. Although leptin was once thought to have a largely central role, it is now known that it affects several physiological processes, such as immune responses, metabolism, and reproduction. This highlights the wide range of tasks that leptin plays in addition to its role in hunger regulation. Leptin has been the subject of extensive research for the past 20 years, and this work has shed light on the complex relationships between nutrition, metabolism, reproduction, immune functions, and inflammation (Pérez-Pérez et al., 2020). It is well known that leptin plays a crucial role in the relationship between nutritional status and the pro-inflammatory T-helper 1 immune response, and a drop in leptin plasma concentration during periods of dietary restriction might affect immunological performance. Additionally, numerous studies have linked leptin to the pathogenesis of chronic inflammation, and elevated circulating leptin levels associated with obesity appear to contribute to the low-grade inflammatory

background that increases the risk of developing degenerative diseases, type II diabetes, and cardiovascular diseases in obese people (Iikuni et al., 2008)



(Havel, 2000)

1.2.1 Leptin and receptor

The leptin molecule is 16 kDa in size and comprises 167 amino acids (including a 21 amino acid secretory signal sequence), and it exhibits the tertiary structure of a globular protein. Leptin acts via its transmembrane receptors, the LEP-R, which exhibit structural similarity to the class I family of cytokine receptors. Both human chromosome 7 (OB) and mouse chromosome 6 (OB) have the obesity gene (Obradovic et al., 2021).

1.2.2 Leptin regulation

Leptin is an important regulator of metabolic, reproductive, and immune functions. Increased levels of leptin gene may play a role in energy homeostasis, neuroendocrine function, angiogenesis, and the production of inflammatory mediators. Indeed, leptin can activate dendritic cells (DC), monocytes, and macrophages and stimulate them to express and release Th1-type cytokines (Obradovic et al., 2021).

1.2.3 Leptin, the adipose tissue

Leptin plays a crucial role at the intersection of metabolism and inflammatory responses. Its production by adipose tissue facilitates the secretion of proinflammatory cytokines like TNF, IL-1, and IL-6, which, in turn, stimulate the release of leptin from adipocytes. In cases of high body fat mass, such as obesity, there is an increase in leptin levels. This surplus of leptin contributes to inflammation and the expansion of effector T cells (Teff), while also restricting the proliferation of regulatory T cells (Tregs). Conversely, decreased leptin levels promote Treg activity but are associated with a reduced number of Teff. The ability of this molecule to generate or sustain low-grade inflammation that may ultimately favor the development of chronic disease is highlighted by leptin's activity and its strong relationship with various pro-inflammatory mediators. Given that leptin levels are normally increased with obesity, this may occur more frequently (Iikuni et al., 2008).

Proinflammatory cytokines, including TNF-, IL-6, and IL-1, that are produced locally in the endothelium by tissue macrophages and circulate in the body are hypothesized to have a role in the atherogenic process. It has been demonstrated that they cause the endothelium to express cellular adhesion molecules, which facilitate the trapping of leukocytes and monocytes

and the start of the atherogenic process (Tsiotra et al., 2013). Leptin is one of these adipokines that is created by adipose tissue. White adipose tissue is the principal producer of this signaling molecule, which is a member of the peptide hormone family (Münzberg & Heymsfield, 2015). Leptin is a cytokine-like hormone that has been shown to regulate innate and adaptive immunological responses, as well as energy expenditure and metabolism.

The activation of natural killer cells, chemotaxis of neutrophils, and secretion of tumor necrosis factor (TNF), IL-6, and IL-12 from macrophages (Francisco et al., 2018) also involve leptin.

1.3 Triphala and classifications

Triphala, a renowned polyherbal medicine derived from the dried fruits of three plant species, namely *Emblica officinalis, Terminalia bellerica*, and *Terminalia chebula*, holds a significant place in traditional Ayurvedic medicine. The term "Triphala" originates from Sanskrit, where "tri" means three and "phala" refers to fruits. Native to the Indian subcontinent, Triphala has been widely used for over a thousand years, as documented in ancient Ayurvedic texts such as the Charaka Samhita and Sushruta Samhita.

Considered a tridoshic rasayana in Ayurveda, Triphala is believed to possess rejuvenating and longevity-promoting properties that benefit individuals of various constitutions and age groups. The formula combines three fruits, namely Haritaki, Bibhitaki and Amalaki (Indian Gooseberry), usually in equal proportions. Its traditional use involves consuming Triphala with honey and ghee on a daily basis. According to the writings of Charaka, a prominent Ayurvedic physician, regular use of Triphala Rasayana has the potential to confer a life devoid of old age and diseases, enabling individuals to reach a remarkable lifespan of one hundred years (Peterson et al., 2017). The partially matured fruit of *T. belerica* and the outer covering of *T. chebula* fruit have been documented to possess purgative properties (Vani et al., 1997). The fruit of *T.chebula* was traditionally used to cure asthma, urinary disorders, heart disease and it has cardiotonic activity (Reddy & Reddanna, 2009). In-depth research on this formulation has revealed a wide range of biological activities, including antioxidant, anticancer, antidiabetic, antiproliferative, antimutagenic, and radioprotective properties (Kaur et al., 2005). In the Ayurvedic system of medicine, the fruit of *Emblica officinalis* is traditionally used for its beneficial effects on the cardiovascular system, brain function, and digestive health. Additionally, it has been recognized for its potential anticancer properties. This fruit is notably abundant in vitamin C, a renowned

antioxidant. Furthermore, studies have shown that the crude extract of *E. officinalis* possesses antioxidant properties, which may help mitigate the harmful effects of certain metals on the liver and kidneys (Singh & Kumar, 2021). Triphala has been found to have radio -protective effects in mice exposed to gamma radiation. *Terminalia belerica* is reported to protect myocardial necrosis reduces cholesterol-induced atherosclerosis and acts as hepatoprotective (Mukherjee et al., 2006).

This formulation, which is high in antioxidants, is crucial for the treatment of a wide range of ailments, including infections, obesity, anemia, fatigue, constipation, and infectious diseases including tuberculosis, pneumonia, and AIDS (El-Mekkawy & Merely et al, 1995). Additionally, preliminary research on Triphala's effects on arthritis-induced mice has demonstrated its anti-inflammatory, immunomodulatory, and lysosomal membrane stabilizing properties (Prince Sabina et al., n.d.).

Gallic acid has been reported to be present in Triphala. It is a phenolic component of plant origin and is chosen as a bioactive marker for these fruits. It is easily available and is studied for its anti-obesity properties. Among the fruits, *Emblica officinalis*. has the highest levels of gallic acid (Gautam A et al., 2008).

1.3.1 Triphala Uses

Anti-inflammatory effects of Triphala:

Triphala has demonstrated potential as an anti-inflammatory drug. In research, Triphala outperformed or was equally effective as a standard pharmaceutical treatment for a range of biochemical indicators of inflammation. Additionally, Triphala significantly reduced the degeneration of bone and cartilage as well as inflammatory indicators in arthritic rats. The expression of inflammatory mediators was decreased by Triphala (Peterson et al., 2017).

Triphala in gastrointestinal health:

Triphala has gained significant recognition for its role in promoting gastrointestinal health. Animal studies have demonstrated that Triphala extracts, whether aqueous or alcohol-based, possess the ability to prevent diarrhea. Moreover, Triphala exhibits entero-protective effects, which can be attributed to its rich antioxidant content. In a mouse model, Triphala demonstrated a reduction

in colitis symptoms, an outcome attributed to its antioxidant properties and high concentration of flavonoids (Peterson et al., 2017)

1.4 RAW 264.7 cell line

The RAW 264.7 cells are monocyte/macrophage-like cells produced from a BALB/c mouse cell line that has been transformed by the Abelson leukemia virus. RAW 264.7 is a renowned mouse macrophage cell line for use in laboratory studies of immune responses and inflammation. The immune system relies largely on macrophages, and when they get activated, a number of inflammatory mediators can be released. In drug discovery and screening research, the RAW 264.7 cell line is frequently employed to evaluate the efficacy and potential side effects of novel chemicals or therapeutic treatments. These cells might shed light on medicines' immunomodulatory effects and effects on inflammation. These cells are referred to as an accurate macrophage model as they are capable of phagocytosis and pinocytosis. Nitric oxide (NO) production and phagocytosis are both increased in RAW 264.7 cells in response to LPS stimulation (Taciak et al., 2018). Previous studies have used the well-known RAW 264.7 macrophage cell line to assess whether Triphala extract can reduce inflammation in macrophages (in vitro) driven by bacterial LPS (Wang et al., 2012).

This study of the anti-inflammatory effect of Triphala extract in leptin-induced inflammation in the RAW 264.7 cell line is significant because it may provide insight into the potential therapeutic benefits of Triphala in controlling inflammation associated with diseases. Thus, we aim to provide a preclinical evidence to validate the anti-inflammatory effect of Triphala extract in leptin-induced inflammation.

2. MATERIALS AND METHODS

2.1 Cell culture

RAW 264.7 murine macrophage cells were obtained from the National Centre for Cell Science (NCCS). The cells were cultured in Dulbecco Modified Eagle Medium (DMEM) with high glucose, supplemented with 10% fetal bovine serum, 100 g/ml of penicillin, and 100 g/ml of streptomycin. Additionally, 2% glutamine was added to the medium as a supplement. The cell culture was maintained at a temperature of 37°C in a humidified atmosphere containing 5% CO_2.

2.2 Reagents

Fetal bovine serum(gibco), penicillin/streptomycin, and DMEM(HIMEDIA), Trypsin EDTA(HIMEDIA), L-Glutamine (HIMEDIA), DMSO (HIMEDIA), Trypan blue (HIMEDIA), MTT – (Sigma Aldrich)

Glass apparatus: 96 well plates (FALCON), 24 Well plate (FALCON)

2.3 Maintenance of cell line

The culture medium was removed from the RAW 264.7 cell line flask, followed by the addition of 1 ml of PBS for thorough washing. This process was repeated twice. Subsequently, 1 ml of Trypsin was added to the same culture flask. After 50 seconds, the Trypsin-PBS-Versene-Glucose (TPVG) solution was removed, and the flask was placed in an incubator for 15 to 20 minutes, allowing the cells to detach. The culture flask was then observed under a microscope to ensure detachment. Next, 4 ml of fresh media was added to the culture flask and mixed well. A new culture flask was prepared, and 2 ml of the previously prepared cell suspension was transferred to the new flask. Both flasks were supplemented with 5 ml of fresh media, resulting in a total volume of 7 ml in each flask. The flasks were subsequently placed in an incubator for further incubation.

Figure. 2.3.1: RAW 264.7 cell line

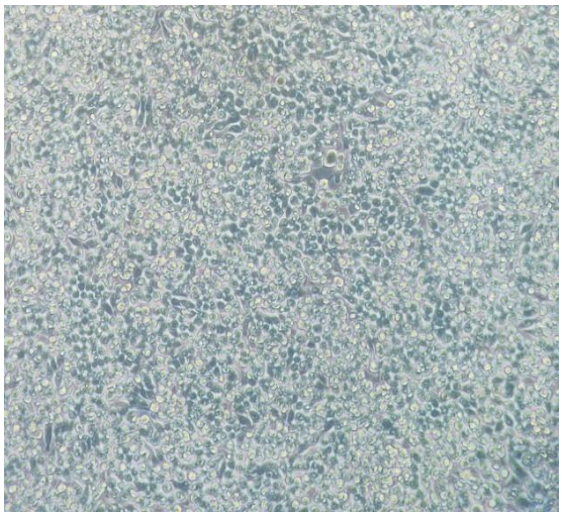


Figure. 2.3.2: Microscopic view

2.4 Cell counting

The media was aspirated from the flask containing the RAW 264.7 cell line. Subsequently, 1 mL of phosphate-buffered saline (PBS) was added to the culture flask, thoroughly mixed, and then removed through repeated washes with PBS. Next, 1 mL of trypsin was added to the same culture and incubated for 50 seconds before removing the trypsin-PBS mixture. The flask was then placed in an incubator for 15 minutes to allow for detachment of the cells, which were observed under a microscope. After confirming cell detachment, 4 mL of fresh media was added to the culture flask and mixed well. For cell counting, a 100 µL cell suspension was mixed with 100 µL of trypan blue, and the resulting mixture was loaded onto a hemocytometer. The total number of cells, as well as the average number of cells, was determined to be 2*10^4. Finally, the cell suspension was seeded into a 96-well plate, with each well receiving 180 µL of the suspension.

Figure. 2.4.1. Neubauer Chamber

2.5 Triphala drug extract preparation

Triphala churna was obtained from Pharmanza Herbal Pvt Limited. A total of 0.36 g of Triphala churna was weighed to prepare a concentration of 10 mg/mL of Triphala extract. To dissolve the extract, 6 ml of distilled water was added to the weighed Triphala churna and mixed thoroughly using a vortex machine to ensure proper dispersion of the extract. The mixture was then subjected to centrifugation at 2000 rpm for 10 minutes to separate the supernatant from any solid particles. The resulting supernatant was carefully collected, ensuring the exclusion of any sediment or debris.

To determine the concentration of the Triphala extract, 1 ml of the supernatant was added to a petri plate and incubated for 24 hours to allow for complete evaporation of the liquid component. After incubation, the petri plate was weighed to measure the weight of the dried extract. The concentration of the extract was calculated as 49 mg/mL based on the weight of the extract obtained from 1 ml of the supernatant.

The yield for the desired concentration of 10 mg/mL was determined using the formula $N1V1 = N2V2$, where N1 represents the concentration of the stock solution, V1 represents the volume of the stock solution used, N2 represents the desired concentration, and V2 represents the desired volume. By substituting the values, the appropriate volume of the extract was determined.

Figure 2.5.1: Triphala Extract

Figure. 2.5.2 : Triphala Extract

Figure. 2.5.3: Supernatant collection

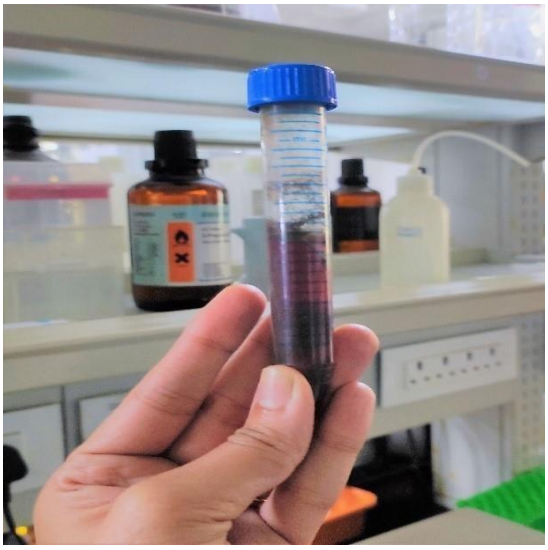


Figure. 2.5.4: Supernatant collection

Calculations-

Extract yield = Evaporate petri plate - empty petri plate

= 4.6869 - 4.6416

Given = 0.0453mg/mL

Required = 10mg/mL

N1V1 = N2V2

45.3 * x = 10*1

= 10mg/mL*1/45.3mg/mL

= 10/45.3

= 220µl+780 pbs

= 1000µl (10mg/mL)

2.6 MTT assay

2.6.1 Cell viability on Triphala extract

RAW 264.7 cells were seeded into 96-well plates at a density of 2×10^6 cells/well and cultured in Dulbecco Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 µg/mL penicillin, 100 µg/mLstreptomycin, and 2% glutamine. The cells were incubated at 37°C in a 5% CO_2 humidified incubator for 24 hours to allow attachment.

After the cells had attached, different concentrations of Triphala extract (15.625 µg/ml, 31.25 µg/ml, 62.5 µg/ml, 125 µg/ml, 250 µg/ml, 500 µg/ml, 1000 µg/ml) were added to the wells. The cells were then incubated for an additional 48 hours.

Following the incubation period, the MTT assay was performed. A 0.5 mg/mL MTT dye solution was added to each well, and the plates were wrapped in aluminum foil to protect from light. The cells were incubated for 4 hours at 37°C and 5% CO_2 to allow the formation of formazan crystals. After the incubation, the medium was removed, and the formazan precipitate was solubilized in dimethyl sulfoxide (DMSO). The absorbance of the solubilized formazan was measured at 550 nm using a Microplate Reader. DMSO was added to the wells to facilitate solubilization.

Figure. 2.6.1: Cells Seeded in 96 well plate

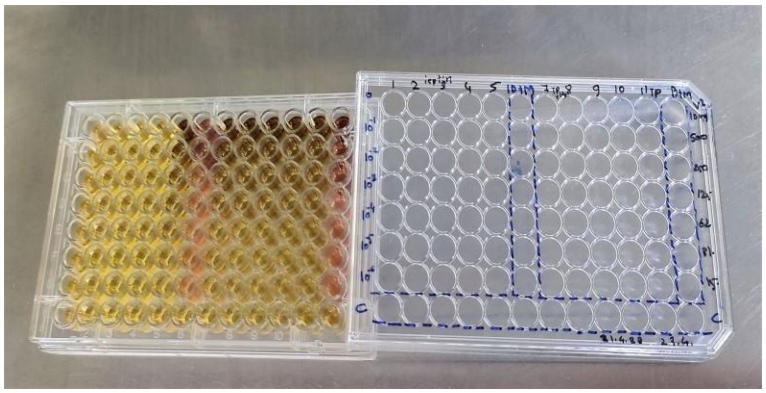


Figure. 2.6.2: After the addition of MTT dye

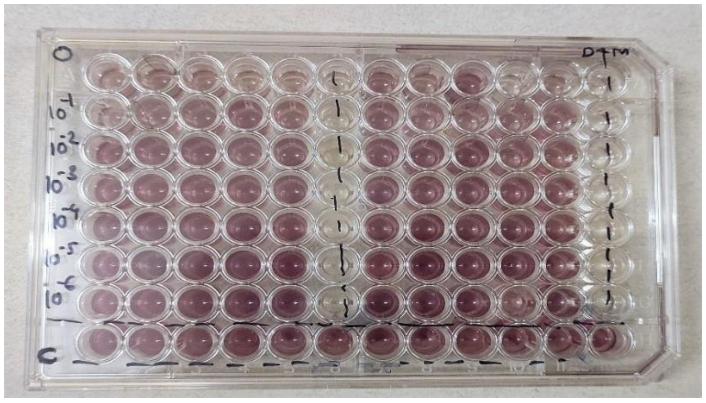


Figure. 2.6.3: After the addition of DMSO

2.6.2 Cell viability on leptin

RAW 264.7 cells were seeded into 96-well plates at a density of 2 × 10^6 cells/well and cultured in Dulbecco Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 µg/ml penicillin, 100 µg/mL streptomycin, and 2% glutamine. The cells were incubated at 37°C in a 5% CO_2 humidified incubator for 24 hours to allow attachment.

After the cells had attached, different concentrations of leptin (1000 ng/mL, 500 ng/mL, 250 ng/mL, 125 ng/mL, 62.5 ng/mL, 31.5 ng/mL, 15.625 ng/mL) were added to the wells. The cells were then incubated for an additional 48 hours.

Following the incubation period, the MTT assay was performed. A 0.5 mg/mL MTT dye solution was added to each well, and the plates were wrapped in aluminum foil to protect from light. The cells were incubated for 4 hours at 37°C and 5% CO_2 to allow the formation of formazan crystals. After the incubation, the medium was removed, and the formazan precipitate was solubilized in dimethyl sulfoxide (DMSO). The absorbance of the solubilized formazan was measured at 550 nm using a Microplate Reader.

Figure. 2.6.4: MTT Assay

2.7 Preparation of standard sodium nitrite

Weigh 6.895 mg of Sodium Nitrite and dissolve it in 1 ml of distilled water (D/W)

Prepare serial dilutions from a 100 mM stock solution to obtain concentrations of 100 µM, 80 µM, 60 µM, 40 µM, and 20 µM. Use the medium as a blank, which will be dissolved with Dulbecco Modified Eagle Medium (DMEM).

Thoroughly vortex the solutions to ensure proper mixing.

Take a 96-well plate and label it accordingly. After a 10-minute incubation period, measure the Optical Density (O.D.) at 520 nm using an ELISA plate reader.

2.8 Nitric oxide assay

Nitric oxide (NO) is a paramagnetic radical gas that exhibits a strong affinity for binding to ferrous hemoproteins, including soluble guanylate cyclase and hemoglobin. Nitric oxide is a gas that acts as a signaling molecule in various physiological processes, such as vasodilation, immune response, and neurotransmission.

Griess assay: This method involves the reaction of nitrite with sulphanilamide and N-(1-naphthyl) ethylenediamine dihydrochloride to form a pink azo compound. The absorbance of the colored product is measured at 520 nm and is proportional to the amount of nitrite present, which is an indirect measure of nitric oxide.

Method -

RAW 264.7 cells were cultured in 24-well plates at a density of 5 × 10^5 cells per well in triplicates and incubated for 24 hours. Following the incubation period, leptin and leptin + Triphala combined were added to the respective wells. After an additional 24 hours of culture, the supernatant was collected and used as a measure of nitric oxide (NO) production. The collected supernatant (100 µl) was mixed with an equal volume of Griess reagent, and the absorbance was measured at 520 nm. The concentration of nitrite was determined by constructing a standard curve using DMEM. The relative cell viability of each experimental group (n=2) was expressed as a percentage compared to the no-addition (NA) control, while the untreated controls were considered 100% viable.

Figure. 2.8.1 cells Seeded in 24 well plate

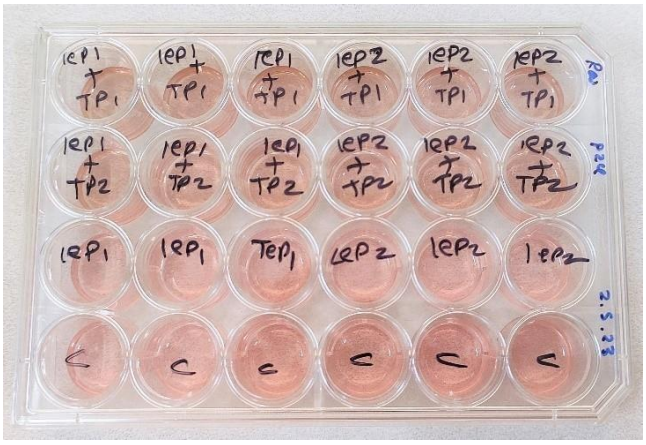


Figure. 2.8.2 After 24 hrs. of drug addition

Figure. 2.8.3: Supernatant collection after 24 hours

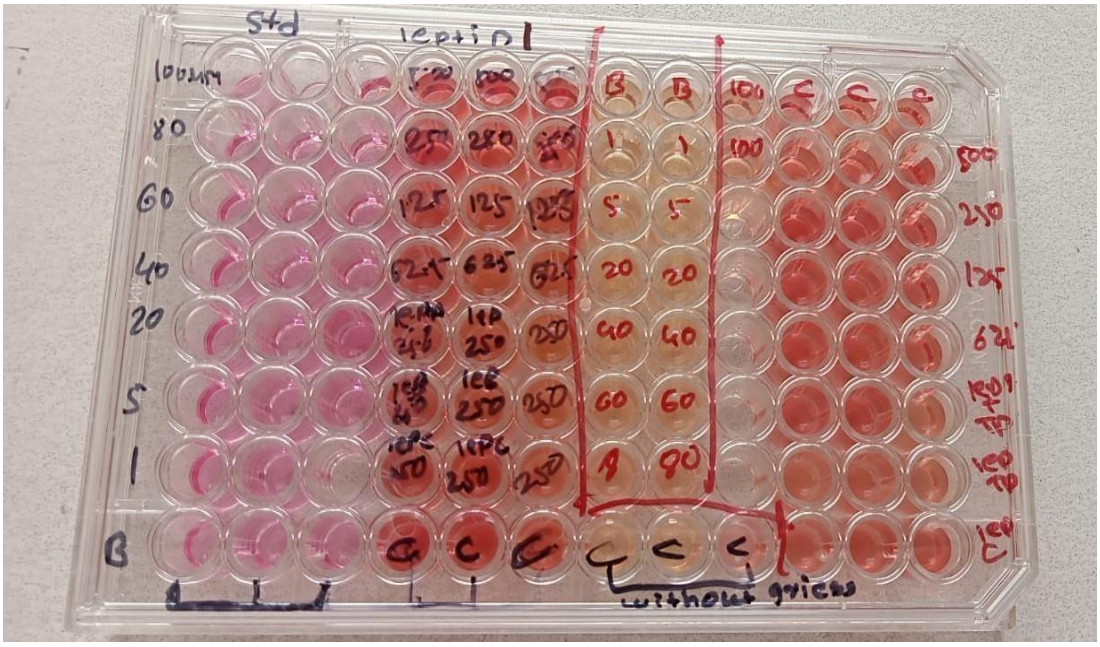


Figure. 2.8.4: 96 well plate for NO production

3. RESULTS AND DISCUSSION

3.1 Effect of Triphala extract on cell viability of raw 264.7 cell line

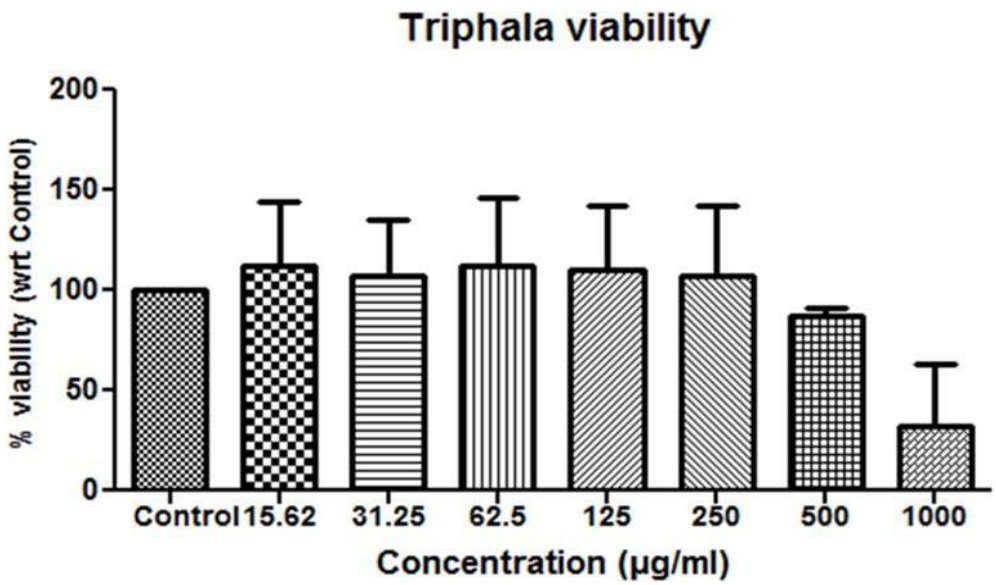


Figure. 3.1

Figure. 3.1 shows that, RAW 264.7 cells were treated with Triphala extract to determine the viability of the cells. Additionally, the findings indicate that a higher concentration of Triphala extract is hazardous to cells and causes cell death. Cell viability was seen to decline at a concentration of 1000 g/ml.

P value < 0.05

3.2 Effect of Leptin on cell viability of raw 264.7 cell line

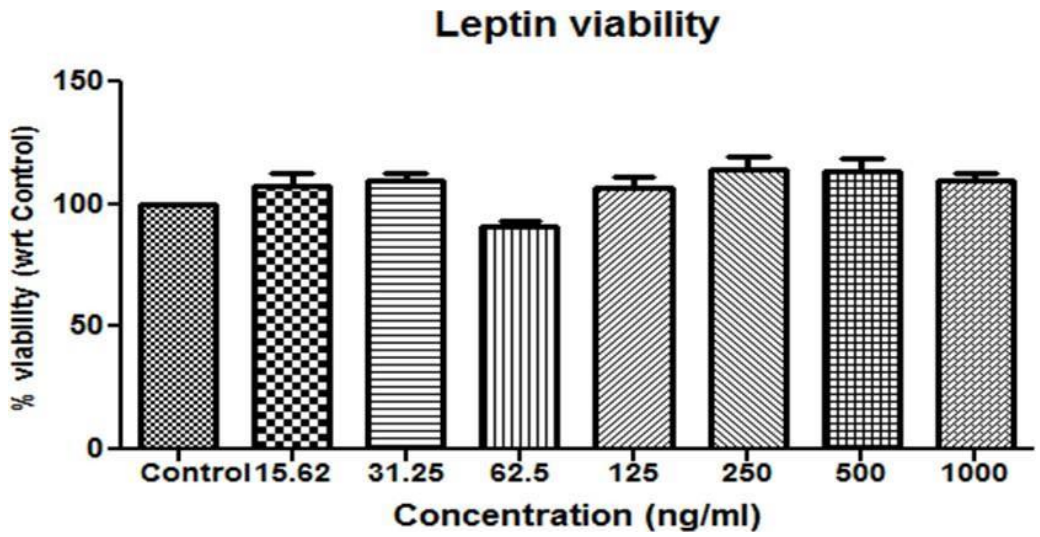


Figure. 3.2

Figure 3.2 shows that leptin does not shows any effect on cell viability of Raw 264.7 cell line.

P value > 0.05.

3.3 Standard sodium nitrite curve

Std. Sodium Nitrite concentrations was prepared by dilutions 20µM, 40µM, 60µM, 80µM, 100µM.

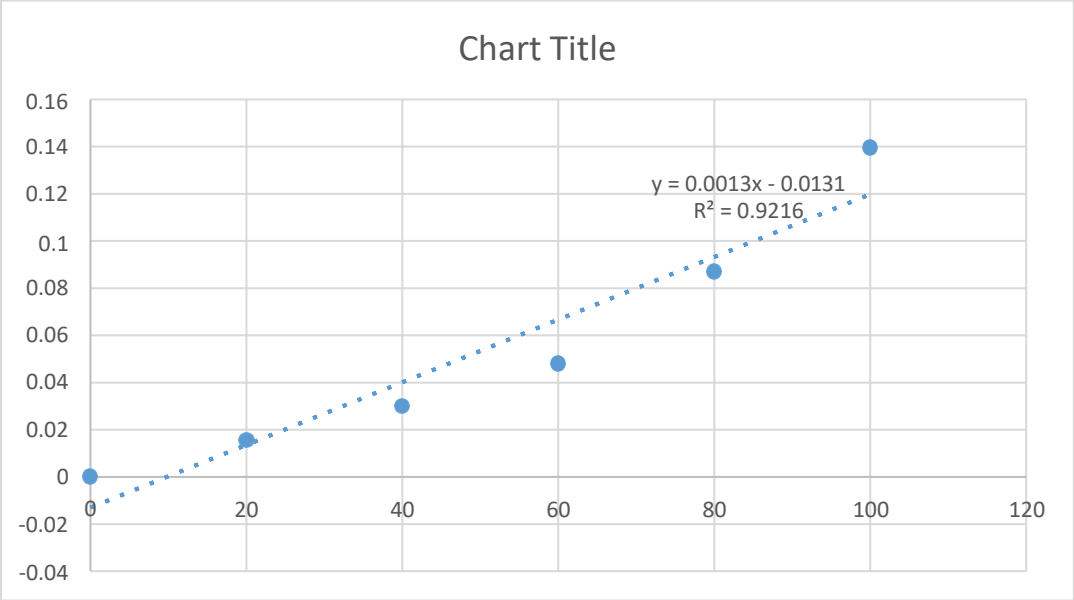


Figure. 3.3

The linear regression equations for nitrite standard curve were calculated as y=0.0013x-0.0131 R^2= 0.9216)

3.4 Nitric oxide (NO) assay results

3.4.1 Effect of Leptin in raw 264.7 cell line

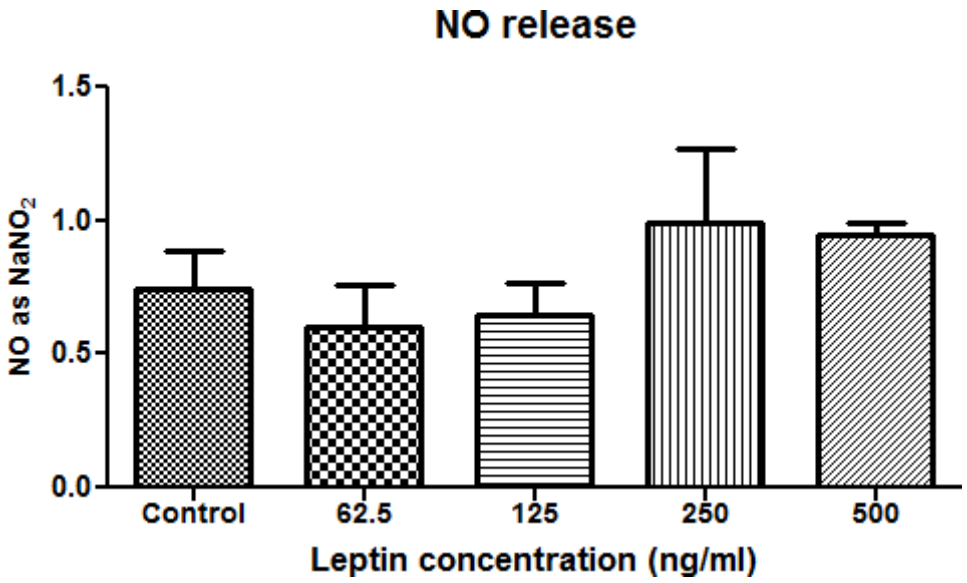


Figure. 3.4.1

Figure 3.4.1 shows that to study the effects of leptin on macrophages, leptin was applied at increasing doses to RAW 264.7 cells for 24 hrs. Supernatants were collected, treated with Griess reagent, and analysed by NO production. The present study reported leptin-induced inflammation in the Raw 264.7 cell line. P value < 0.05

3.4.2 Effect of Leptin and Triphala extract in raw 264.7 cell line

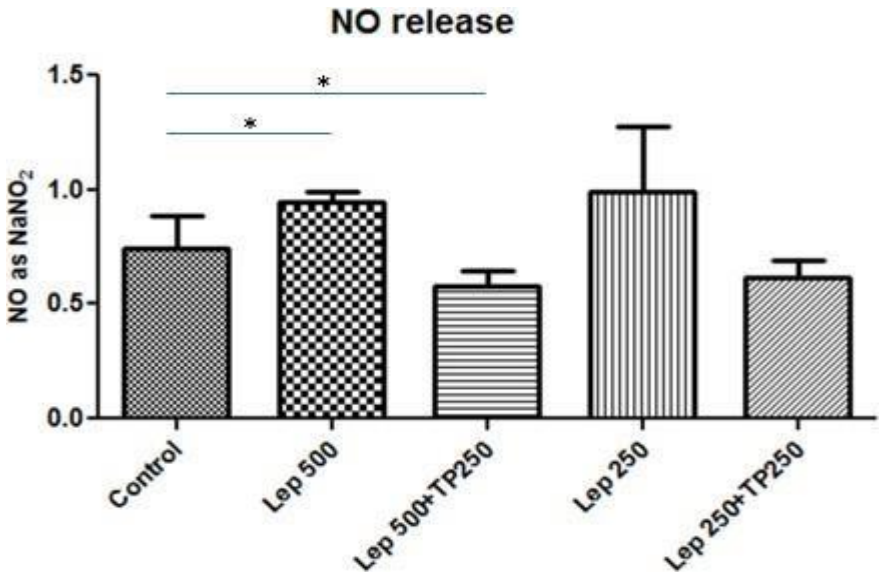


Figure. 3.4.2

In this figure 3.4.2, it is shown that leptin 500 ng/mL shows leptin-induced inflammation in NO production. Leptin 500 ng/mL and Triphala 250 g/ml combined show a reduction in inflammation. Because of the high standard deviation, leptin 250 ng/mL does not show inflammation in NO production. An unpaired t test was conducted. P value < 0.05

To explore anti-inflammatory molecular mechanisms, the current study focused on the inhibitory effects of the Triphala extract on leptin induced inflammation in RAW 264.7 cell line. Based on the results obtained in this study, it is evident that leptin plays a significant role in inducing inflammation in the RAW 264.7 cell line. The concentration-dependent increase in inflammation observed with increasing doses of leptin highlights its pro-inflammatory effects. Furthermore, the combination of leptin and Triphala extract demonstrated a reduction in inflammation, indicating the potential anti-inflammatory properties of Triphala.

The observed reduction in inflammation induced by leptin and its subsequent mitigation by Triphala extract highlight the potential of Triphala as an effective agent in reducing inflammation. Moreover, the evaluation of nitric oxide (NO) release revealed that Triphala extract effectively reduced NO production, indicating its ability to modulate the inflammatory response. These findings align with previous reports highlighting the anti-inflammatory effects of Triphala in various experimental models. Additionally, the observed effects on cell viability further emphasize the potential of Triphala extract as a safe and viable option for managing inflammation. The lack of significant effects on cell viability suggests that Triphala extract exerts anti-inflammatory effects without compromising cell viability.

Earlier reports showed that Triphala herbal extract suppresses inflammatory responses in LPS-stimulated RAW 264.7 macrophages and adjuvant-induced arthritic rats via inhibition of NF-κB pathway" investigates the anti-inflammatory effects of Triphala herbal extract. The study focuses on examining the response of Triphala extract in reducing inflammation in both cellular and animal models. The findings demonstrate that Triphala extract effectively inhibits inflammatory responses in LPS-stimulated RAW 264.7 macrophages and adjuvant-induced arthritic rats by targeting the NF-κB pathway (Kalaiselvan & Rasool, 2016).

The active principles of Triphala, namely chebulagic acid, chebulinic acid, and gallic acid, were shown to effectively inhibit the pro-angiogenic and pro-inflammatory activities induced by TNF. This inhibitory effect was attributed to the suppression of p38, ERK, and NFB phosphorylation. These results further support the anti-inflammatory properties of Triphala and its potential as a therapeutic agent for managing inflammatory and angiogenic disorders (Shanmuganathan & Angayarkanni, 2018).

4. CONCLUSION

- Findings of this study indicate that the concentrations of Triphala and leptin utilized did not exert a significant impact on cell viability in the RAW 264.7 cell line. However, the results clearly demonstrate the dose-dependent induction of inflammation by leptin, reaffirming its role in promoting inflammatory processes.

- On the other hand, Triphala extract demonstrated potent anti-inflammatory effects by effectively reducing leptin-induced inflammation and inhibiting NO release in the RAW 264.7 cell line. These results highlight the potential of Triphala as a promising therapeutic agent for mitigating inflammation associated with leptin-mediated processes.

- One of the constraints faced was the limited time period available for research. Due to this, it was not possible to investigate the effects of Triphala extract on chronic inflammatory conditions thoroughly. Exploring the impact of Triphala extract on such conditions would require a more extensive study conducted over a longer period of time. Therefore, it is recommended that future studies consider a more extended timeframe to comprehensively evaluate the effects of Triphala extract on chronic inflammatory conditions.

- The outcomes of this study contribute to a better understanding of the role of Triphala and leptin in inflammatory responses and pave the way for future studies focused on exploring novel therapeutic strategies targeting inflammation and its associated complications.

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